ROUTINE EXAMINATION OF THE CEREBROSPINAL FLUID IN SUSPECTED SYPHILIS OF THE NERVOUS SYSTEM; RECENT MODIFICATIONS IN THE PREPARATION OF LANGE'S COLLOIDAL GOLD SOLUTION.*

By THOMAS G. INMAN, San Francisco.

Investigation of the cerebrospinal fluid as an aid in the diagnosis of diseases of the central nervous system has, during the past ten years, assumed a position of the greatest importance. In not a few diseases a correct interpretation of the physical or chemical changes in that fluid, or the detection of a bacterial intruder, will determine the nature of the disorder in question with a degree of certainty not afforded by any other means at our command. Thus lumbar puncture has become almost a routine practice in the neurological examination.

It is, however, to the diagnosis of those diseases of the central nervous system caused by syphilis that modern refinements in serological examination have brought the greatest aid supplementing the clinical and blood findings, or, when the former are doubtful and the latter negative, often furnishing the necessary evidence upon which a positive diagnosis can be made. The occasional failure of some or of all of these laboratory helps detracts but little from their great value. It is enough to remember that failures may occur.

The now universal employment of lumbar puncture in suspected syphilis of the central nervous system is sufficient evidence that the value of this procedure is well recognized but there is another service which may be rendered by this practice of equal if not of greater value than its use in suspected disease. I refer to the systematic examination of the cerebro-spinal fluid at the close of treatment in every case of lues to determine if possible the existence of a central nervous system infection which we now know may persist after the systemic symptoms have disappeared and the blood findings have become negative.

Should this examination show involvement of the nervous system, further anti-syphilitic treatment may secure complete abolition of the disease, prevent further advance to a paresis or tabes and at the same time destroy a focus which might later reinfect the general circulation.

This communication has for its object the presentation of a brief description of a satisfactory routine method of examining the cerebro-spinal fluid in these cases and to urge a more general acceptance of this or of any other good method, in an effort to determine in as far as it is possible to do so, the integrity of the central nervous system before dismissing, as cured, a patient who has been undergoing treatment for syphilis. In the words of Professor Wechselmann, "A lues therapy which neglects a concluding examination of the cerebro-spinal fluid must be looked upon as worthless."

The method here outlined is the one in use in Professor Wechselmann's division of the Virchow Krankenhaus, Berlin, and to his assistant, Dr. Hans Eicke, I am indebted for some of the accompanying details and for the opportunity of testing the gold solution on many spinal fluids.

The different procedures are:

1st. The Nonne-Apelt test with ammonium sulphate solution commonly known as phase 1.

2nd. The Roberts-Stolnikow-Brandberg quantitative test for whole albumen as modified by Pfaundler for the spinal fluid.

3rd. Estimation of the cell content quantitatively.

4th. Wassermann complement fixation test.

5th. Lange's colloidal gold reaction.

The cerebro-spinal fluid 6 to 8 c. c. is received in two test tubes—the first specimen, the one most likely to be contaminated with blood corpuscles, is used for the Wassermann, the second is reserved for the other tests. Blood corpuscles may be removed by centrifugalizing the fluid at once in an electric centrifuge for twenty minutes.

Nonne-Apelt: To .5 c. c. of the fluid in a clean polished test tube .5 c. c. of a saturated solution of neutral ammonium sulphate made with boiling water and filtered, is added. The tube is shaken, allowed to stand for at least three minutes and examined by holding it before a black background and allowing the light to penetrate obliquely from above. The different degrees are described as: 1, negative—no darkness, no opalescence; 2, trace of opalescence; 3, opalescent; 4, cloudy; 5, marked cloudiness.

One may first make the Ross-Jones ring test by gradually adding the fluid to the ammonium sulphate solution, a ring of hair-like fineness denoting an increase in globulin. After observing the ring, shake the mixture and have the Nonne-Apelt.

Roberts-Stolnikow-Brandberg-Pfaundler quantitative albumen test. Add to the Nonne mixture 4 c. c. distilled water, the precipitated globulin is dissolved, the solution contains spinal fluid in the proportion of 1:10 and is used in making further dilutions as follows:

To	1 c. c.	add	.5 c. c.	distilled	water	= 1:15
"	1 c. c.	"	1 c. c.	"	"	= 1:20
"	.5 c. c.	"	1 c. c.	"	"	= 1:30

and so on.

These are added (beginning with the weakest so as not to carry over a stronger solution to a weaker one) to an equal number of test tubes containing about 2 c. c. of pure nitric acid, the solution being allowed to flow slowly upon the acid from a fine pipette placed against the side of the test tube at a point just above the surface of the acid.

The tubes are now examined against a black background, as in the Nonne-Apelt test and a record made of the weakest solution showing an opalescent ring as 1:30, 1:40, etc., as the case may be. It is obvious that if the Nonne-Apelt shows a marked cloudiness it is not necessary to make quantitative tests on solutions below 1:30.

Normal fluid is said to show a ring in a dilution of 1:15 corresponding to .2 of albumen per mille. A noticeable ring with dilutions above this must be considered pathological.

* Read before the San Francisco County Medical Society, January 5, 1915.

The Colloidal gold test, suggested by the known action of solutions of proteid substances upon colloidal gold solutions was described in 1912 by Carl Lange of Berlin. He found that spinal fluid from patients suffering from syphilitic diseases of the central nervous system gave a characteristic reaction, denoted by a color change in certain dilutions. In fluids from cases of tuberculous meningitis or acute inflammatory affections of the meninges a color change in different dilutions occurred.

Numerous observers have substantiated his findings and with few exceptions have declared that we have in this reaction a valuable adjunct to other methods of serological examination now in use. The method, however, is yet in a transitional stage. The formula given here and the manner of preparation are somewhat different from that in general use, grape sugar being substituted for the formalin. The results seem to warrant the change as the new solution appears more specific in its action and is quite stable.

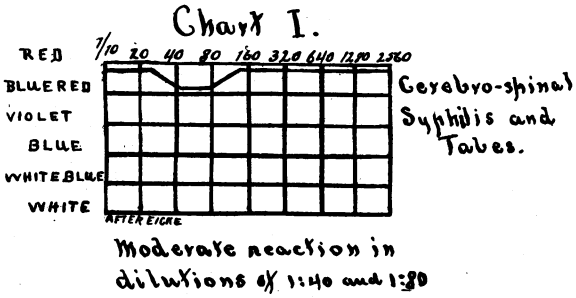

CHART I

The following description of this reaction is purposely abbreviated as there are many full discussions of the processes of preparation and application in recent literature. For the preparation of the colloidal gold solution proceed as follows:

Into a perfectly clean 1000 c.c. Florence flask put 500 c.c. fresh doubly distilled water, 5 c.c. of a 1% solution gold chloride and 2.5 c.c. of a 5% solution grape sugar (Mercks water free). Bring quickly to boiling and at once add 3 c.c. of a 3% solution potassium carbonate. Within a few seconds the solution becomes a bright, clear, red color, and is removed from the fire. If contaminated distilled water, impure chemicals or new or soiled glassware is used, the fluid is not transparent or shows a tinge of purple and must be rejected.

The solution should always be tested on a spinal fluid from a case of known paresis and on a blood serum mixture in the proportion of .1 c.c. normal blood serum to 5 c.c. normal spinal fluid.

The success of the preparation of the gold solution depends on the use of pure doubly distilled water, chemically pure chemicals and absolutely clean utensils, preferably of Jena glass.

In a test tube rack place 10 test tubes. In the one to the extreme left place 1.8 c.c. of a .4% sodium chloride solution and in each of the other tubes put 1 c.c. of the same solution; .2 c.c. of the spinal fluid is put in the first tube and with a clean pipette thoroughly mixed; 1 c.c. of the mixture is transferred to tube number two, mixed and 1 c.c. removed to tube number three, and so on throughout the series of ten tubes. The extra c.c. left over from the tenth tube is rejected. The tubes now contain spinal fluid in the proportions of 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, 1:5120.

Now to each of the tubes add 5 c.c. of the colloidal gold solution, shake the mixture and set aside for examination later.

No definite time can be set for the appearance of the reaction. With some solutions it begins at once, but in all cases the tubes should be set aside over night and reexamined the following morning.

A positive reaction is denoted by a color change beginning in luetic diseases in the tubes containing dilutions in the proportion of 1:40 and 1:80 as shown in chart one. The change in color is at first slight, beginning as a mild fading of the red color and proceeding through bluish red, violet, blue, white-blue, to white, that is, to complete precipitation as in chart two.

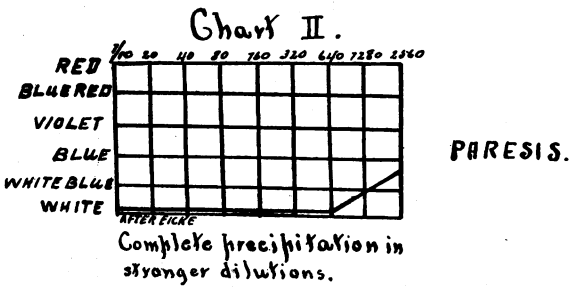

CHART II

The moderate reaction of chart one is that found in syphilis in the secondary stage with beginning changes in the central nervous system and some forms of cerebro-spinal syphilis and tabes. The complete change to white is the reaction seen in general paresis.

In tuberculous meningitis or in forms of meingitis associated with the presence of pus in the spinal fluid the reaction is seen in the weaker dilutions beginning at about 1:320 and extending to the right through three or four tubes; such a case is number three. Sometimes these reactions extend through all the weaker dilutions.

Cytology: As a means of keeping accurate records, the use of a counting chamber to quantitatively determine the cell content is undoubtedly desirable. A diluting fluid consisting of methyl violet .05, glacial acetic acid 0.5 and distilled water c.c. 25. facilitates the operation and at the same time gives some information as to the nature and age of the cells. Cells present in an amount over 10 per c.m.m. may be considered as an abnormal increase. As to the number of cells normally present there is much difference of opinion but as a diagnosis will rarely ever be determined by the cell count alone and since the number of cells present is not necessarily an index of the extent of disease present the number given above is sufficiently accurate for the purpose intended.

Where a counting chamber is not at hand the

French method may be used with satisfactory results. Centrifugalize equal parts of fluid in the usual tubes (2 to 4 c.c. in each tube) for 20 minutes at 2000 revolutions per minute. Drain the fluid off and siphon the deposits into two capillary tubes. Two cover glasses are prepared by drawing on each a small ring with a wax pencil in the center of which the drop in the capillary pipette is deposited. This gives two specimens, one from each centrifuge tube, aids in preventing error and assures somewhat against loss of the specimen during the staining process. The slides are now dried, stained by placing for one minute in May-Grünwald full strength followed by twenty seconds in Giemsa mixture 6 drops to 10 c.c. of distilled water, washed in distilled water and dried. Fifteen fields are counted using a 1/12 oil immersion lens and the average taken; 0.2 cells represents the normal; 3-5 border finding; 8-15 weak positive; 20 or more positive.

The Wassermann must be performed according to the method in use in the laboratory where the work is done.

Of the five tests mentioned a positive Wassermann is undoubtedly the most certain evidence of syphilitic disease of the central nervous system.

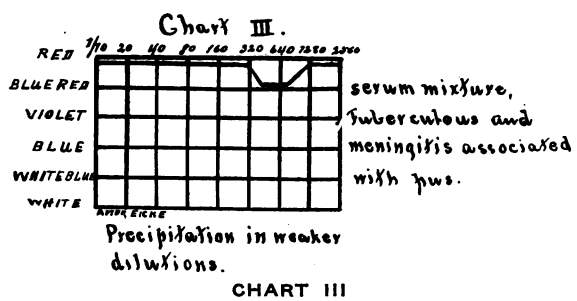

CHART III

But in the first and second stages a positive Wassermann is not often obtained even when cell and protein content are increased. It is here that the gold test will prove a valuable addition to the cytologic and protein determinations. In the spinal fluid of 136 luetics at the Virchow, Eicke found 60 giving a positive gold reaction, moderate lymphacytosis and protein increase though only 4 of the whole number showed a positive Wassermann in the spinal fluid.

It is not the intention to convey the idea that all cases in the first and second stages of syphilis showing changes in the spinal fluid are going to advance to more definite disease. Examination of the spinal fluid in the early stages of syphilis by different observers show that in over 50% there is some change as shown by increased lymphocyte and protein content. Undoubtedly many of these return to normal with or without treatment, but it may be stated as a general truth that if after proper anti-syphilitic treatment, the blood showing a negative Wassermann, the finding of an altered spinal fluid is evidence that the patient is not yet cured.

General paresis and tabes are among the most malignant results of syphilitic infection and are the most difficult to influence favorably by treatment. The very nature of these destructive processes does not allow us to hope from treatment for anything more than an arrest of the disease, some amelioration of symptoms and perhaps a return to normal of those cellular elements not yet wholly destroyed. The time to treat paresis and tabes successfully is as soon after the appearance of the primary syphilitic manifestation as treatment can be applied.

Every individual affected by syphilis in the primary or secondary stages should be looked upon as a potential paretic or tabetic and he has not received the full benefit of all that medicine has to offer him preventively or therapeutically until his case has been proved up by some such method as is here outlined and both blood and spinal fluid can be pronounced normal.

Discussion.

Dr. W. F. Schaller: I have always thought that cerebro-spinal fluid analysis to the neurologist was comparable to urine analysis in a medical case to the internist. The different tests that Dr. Inman has spoken of, with the exception of the Lange colloidal test, are tests we make generally. In speaking of the total albumen tests, I use the nitrate acid test that Sicard (?) described. Like all qualitative tests, it depends upon personal interpretation. There is a diaphanometric test of Mestrezat which I tried to use once, but gave it up. The standard solutions do not keep very well. I think we are in need of some good test for quantitative tests of albumen.

As to the cell count, Dr. Inman tells us that 10 cells is considered probably the upper limit of normal. I certainly agree with that, but I think it is rather high. I estimate six cells as highest for normal fluid. I found two cases in over 100 analyses in which there were six cells in otherwise normal fluid. French observers, in analyzing normal fluids, found that they seldom contain more than three cells to the c.m.m.

I should be pleased to have Dr. Inman tell us more about this Lange test. I know several Boston men, in writing a review of some work done there, considered it more important than increase in cells, and if that is true, it must certainly be important, because I believe you have an actual index of the amount of the meningeal inflammation from the cell count. In spinal cord tumors, we often get a static fluid with increase in albumen and globulin, but a very slight if any increase in cells. Dr. C. M. Cooper has reported a case of this kind. In reviewing cerebro-spinal fluids in cases of tumor, it has appeared to me that I have found the same condition in a number of cases—albumen considerably increased and a very slight increase in cells. In the absence of any signs of syphilis—either clinical or in the Wassermann—I think this is of considerable importance; it has its explanation in the fact that when tumors cause intracranial pressure, we have some stasis and interference with the natural flow of the cerebro-spinal fluid, and get a fluid with an appreciable increase of albumen.

The point Dr. Inman made that in many cases of syphilis it is impossible to tell whether the nervous system has been spared or not without examining the spinal fluid after treatment has been discontinued, is a very timely point. I think this will become a routine test. We know that even in the secondary stage of syphilis we often find an increase of cells in the cerebro-spinal fluid; pain in the back of syphilitics may be from radiculitis due to an extension of the lepto-meningitis of the cord.

Doctor G. Franklin Shiels: I am perfectly convinced of its diagnostic value, but I must register a protest—not against these methods, chemical or microscopical—but against the principle set forth

that lumbar puncture be used as a routine in all cases of syphilis, and in all diseases of the nervous system. Syphilis is so widely spread that one might almost say that the human race is syphilized as well as civilized, and the nervous manifestations of the disease are infinitely few when compared with the number of persons who have, or who have had lues; moreover, the disease is becoming milder and milder as years roll on, and at the same time more curable.

Now since ataxia and paresis are practically recognized as always being of syphilitic origin (the exception, if there be any, proving the rule), and since lumbar puncture must be considered as a major surgical procedure on account of the skill and care with which this operation should be carried out, I protest against it being used except when absolutely necessary, i. e., when the Wassermann blood examination or one of its modifications does not satisfy, and a differential diagnosis is imperative. When used in cases of ataxia or paresis its employment is absolutely indefensible since the disease itself is an absolute diagnosis of the existence of its acknowledged cause. You can see how lightly this procedure is regarded by the remarks of Dr. Schaller, who tells you that he, with most neurologists, considers it just as necessary to examine the cerebro-spinal fluid in the presence of nervous disease, as it is to examine the urine in disease of the kidney. This is terrible. It is always possible that by introducing a needle into the spinal canal a serious injury may be done; suppose, for example, that the danger of such injury were 1/10 of one per cent., and the mortality were 1/100 of one per cent., would you be justified in using this procedure as a part of your routine in diagnosing a case of paresis, or ataxia, when you already knew the nature of the disease and its cause? I think not.

I must not be misunderstood in this matter. I both feel and know that everything which is done in the laboratory which will aid in the most distant way the progress of medical science, or benefit the patient or the people at large (which after all is the sole reason for the existence of our profession), must be valued, and used to the extent of its value.

My contention is that, when you invade any portion of the anatomy, which is vital to the patient, and injure it to the slightest degree, you are putting that patient in jeopardy. I believe that spinal puncture is to be regarded as a serious procedure in which an accident might arise which would be most disastrous to the patient, infection, injury to nerves, etc. While not to be in any way regarded as a similar procedure, except that a needle is introduced into the spinal canal, one instinctively thinks of spinal anesthesia which for a time was lightly regarded, and as lightly used, and vaunted as a harmless substitute for ether, and other anesthetics until experience told all thoughtful surgeons that it had a most serious danger, and should be discarded except in the few rare cases where it might be less dangerous than other means available.

As I have said, lumbar puncture is of aid in making a differential diagnosis in a certain small class of cases where the other diagnostic means available do not suffice, and I again protest against its being used as a routine practice, or to it being referred to as on a par with the examination of the urine either in regard to its necessity, its value, or its freedom from danger.

C. G. Snow: As regards the danger of securing the fluid, I do not agree with Dr. Shiels. I have experienced very little difficulty, and personally I do not regard it as dangerous. As regards its utility, it is not only of value as a positive reaction, but its value is increased as a negative reaction. I can add nothing to Dr. Inman's paper, which was very complete and comprehensive. As regards every case of syphilis being frankly syphilis, I cannot agree with that. I do not believe it is possible to know in every individual case. A patient may have all symptoms of syphilis, may be going through treatment, and may be developing multiple sclerosis. With a spinal puncture you can definitely tell if his nervous symptoms are due to syphilis. As regards the application of the test, it is really easy and I have never seen any false reactions. My personal idea is that it is a more valuable test than the Wassermann when you are determining whether to continue or stop your treatment. When your case is undergoing treatment, it is very interesting to analyze your fluid and watch it go down step by step. As regards the Lange test in association with the other tests, I think that in all spinal fluid examinations you should beware of negative reactions. It has already been noted that in cell counts with tumor of the cord, negative findings are not to be taken as indicating the absence of meningeal irritation. A negative reaction in all spinal fluid examinations except the Lange I am skeptical about. You may have a negative Wassermann—in fact, it is not uncommon to have a negative Wassermann in paresis or tabes in late stages, whereas you would not have a negative Lange. Therefore, I consider it one of the finest tests that have been devised in recent years.

Dr. W. P. Lucas: I have nothing to say except to disagree with Dr. Shiels as to the danger from lumbar puncture. It is done at the Psychopathic Hospital in Boston, where they have 100 to 150 a month, and every case has lumbar puncture except brain tumor cases, and the last report shows no accidents from lumbar puncture. I think, as Dr. Schaller says, that it is absolutely comparable with the urine examination in cases of kidney disease, and is a test that should be carried out on every neurological case.

Dr. G. L. Boalt: May I speak of a case that was sent to me about three years ago for the Wassermann test? The patient was a man twenty-three years of age. The result of the serum examination was a triple plus Wassermann. His physician gave him very thorough treatment with a number of salvarsan and neosalvarsan injections and mercurial treatments in the intervals. After a year and a half he was again sent to me and the blood gave a negative Wassermann reaction, and with frequent examinations during the past eighteen months we have continued to get negative results. A week ago his physician, before dismissing the case, sent the spinal fluid for examination, which gave a triple plus Wassermann. I sent the fluid to Dr. Cummins of the Harriman Research Laboratory at the Southern Pacific Hospital for the Lange's colloidal gold test, who reported that the results suggested an early case of paresis.

I think every syphilitic case treated should have a spinal fluid examination after the disappearance of the secondary svmptoms, and another before being dismissed as cured. Even the possibility of injury to the one person is better than the sorrow of a whole family when that member has been sent from his home to a state hospital too late, and practically incurable.

Dr. Inman, closing discussion: One cannot compare, in as far as the danger attending the procedure is concerned, ordinary spinal puncture for diagnostic purposes with the injection of cocaine or other drugs into the spinal canal. I have seen many spinal punctures without, aside from occasional headache, noting any untoward result.

I agree with Dr. Shiels that it is a major surgical operation in that it must be performed with the same surgical aseptic or antiseptic care which should be preliminary to all operations, major or minor. We prepare as in operating in the abdomen, brain or elsewhere in the body. The puncture field is painted with tincture of iodine, the needle sterilized by boiling and the hands of the operator properly cleansed.

I am not a laboratory worker, but I have brought

these few simple tests together for the use of men who like myself are employed in clinical work. For the trained laboratory man one method may be as good as another and he selects those which best serve his needs. The Nonne, albumen estimation and cell count are probably sufficient to show in most cases whether or not there is any inflammatory change in the central nervous system and for the purposes outlined in this paper that is what is desired.

With regard to the statement of Dr. Schaller that 10 cells per c.m.m. is rather high I would say that this number was taken arbitrarily. I agree with him that for accurate estimation 6-8 would come nearer being the number normally present. I do not think so much reliance should be placed upon the number of cells because with old and young cells present the number is not a definite indication of the extent of the process producing them.

The acetic acid staining mixture aids in differentiating young from old cells but for accurate cytologic work some such method as that of Alzheimer is necessary and this takes too much time for practical purposes.

A sensitive gold solution will show finer syphilitic changes than the Wassermann. The change in the formula is due to an effort to make the reaction more specific, that is, to cause the color change to cling more closely to certain dilutions. This has been shown to be possible to some extent at least in that with this formula the reaction in syphilitic diseases occurs first in dilutions of 1:40 and 1:80.

It was not intended to recommend the indiscriminate performance of lumbar puncture in all cases of known syphilis, but rather, first—to urge its performance at the close of treatment of cases of general syphilis in order to determine that the central nervous system is healthy before dismissing the case as cured, and, second—to outline a systematic method of cerebro-spinal fluid examination which could be easily and safely followed.

AUTONOMIC REFLEXES FROM THE DIGESTIVE TRACT.*

By HARRY B. REYNOLDS, M.D., Palo Alto.

The progress made in diagnosis of diseases of the digestive tract in the last decade is little short of phenomenal. In the past our only avenues of approach were through the subjective history, a physical examination and laboratory studies. The work of Paulow and others working along lines of experimental physiology has formed anew our conceptions of the process of digestion. The studies of Cannon, Hertz, Meltzer and others have accumulated a mass of data on the motility of the digestive canal no less startling than the late discoveries on the heart beat. The Roentgen rays, experimentally and clinically applied, have amassed facts which bring us closer to absolute precision in our diagnosis.

Still another approach lies in a study of visceral neurology. Investigators along special lines have worked out the anatomy of the so-called autonomic system, but its physiology is less clear, being obscured by the intricacies of hormone action, internal secretion, psychic influence and the contradictory evidences of experimentation. It is clear, however, that the various secretory and motor activities of the digestive tube with its accessory glands, while largely local in their origin, are under the general supervision of a nervous mechanism consisting of two factors, the one inhibitory, the other stimulating in its influence.

The activity of this nervous mechanism, however, is not limited to the digestive function. Both the sympathetic and the autonomic systems are concerned with the control of other glands and functions. This circumstance becomes of importance in diagnosis in that the nature of an abnormality in the function of a digestive organ can often be surmised from a concurrent variation in the physiology of the heart, blood vessels, pupils, respiration, etc. Thus the study of these reflexes becomes useful to us both in determining the nature of an abnormality in the digestive tract, and, conversely, in recognizing from a digestive symptom an extra-digestive organic cause.

Another matter well worth study is the very real effect of psychic influence on the functioning of the digestion. To one who only half appreciates this fact the recounting of the experiments of Paulow is very enlightening. We all recognize such outstanding truths as the gastric stasis under emotional strain, the vomiting of a meal during grief, anger or other powerful emotion, but few clinicians give due weight to the influence of prolonged grief, worry, fear, mental depression, discouragement, domestic unhappiness in the causation of constipation, spastic colitis, gastric atony, colonic stasis, and even enteroptosis. Yet a recognition of this very frequent relation will often lead to a cure or the avoidance of a useless nephropexy or colon short-circuiting. When one is confronted by a patient complaining of a digestive disturbance, a little reflection will readily disclose that not all of the symptoms complained of can be directly referred to the lesion discovered but that part of the picture is due to abnormal functioning of distant organs reflexly influenced, while still other complaints may be referred to the psychic state of the individual resulting from subconscious effects of the first two groups of symptoms. Let me illustrate:

Case I. A middle-aged lady, formerly treated for several years for neurasthenia, came complaining of indigestion, vomiting, hunger-pain, tremendous constipation with spastic colitis, frequent bleeding from the bowel, loss of flesh and prostration. An operation for ulcer of the pylorus vastly improved her for two years. Her husband then developed progressing heart disease, during the course of which she devotedly nursed him and during which time she remained perfectly well. Three weeks after his death, unoccupied and depressed, her constipation returned and the mucous colitis again developed in an exaggerated degree. It then became necessary for her to earn her living and, as a means to this end, she took up practical nursing. During her first case, again occupied and forgetful of her misfortunes, she again vastly improved and she has been busy and in excellent health to the present time.

In this case is clearly displayed the relation in the symptom picture of the basic organic lesion, the reflex irritative symptoms and the psychic factor each with its influence in the sum total of symptoms. Moreover, the effect is demonstrated of surgically relieving the proximate cause and of psychically, by occupation and forced interest in the affairs of others, relieving those symptoms built up on the neurosis.

* Read before the San Francisco County Medical Society, April 6th, 1915.